

Combined action of vasoactive amines and bradykinin mediates allergen-evoked thermal hyperalgesia in rats

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Abstract

The ability of allergens to induce hyperalgesia in immunoglobulin E (IgE)-sensitized rats was investigated. The left hind paws of Wistar rats were sensitized with intraplantar injections of IgE anti-dinitrophenylated bovine serum albumin monoclonal antibody, and challenged with dinitrophenylated bovine serum albumin 24 h later. Allergen challenge yielded rapid thermal hyperalgesia and oedema formation in the ipsilateral paws, both reaching a plateau from 15 min to 3 h, and both diminishing thereafter. Allergen-evoked hyperalgesia was inhibited by intraperitoneal treatment with meclizine or methysergide, histamine and 5-hydroxytryptamine receptor antagonists. There was also sensitivity to local treatment with either bradykinin B₁ or B₂ receptor antagonists, des-Arg⁹-[Leu⁸]-bradykinin or D-arginyl-[Hyp³, Thi⁵, D-Tic⁷, Oic⁸]-bradykinin (Hoe 140). Anaphylactic hyperalgesia was mimicked by the combined administration of histamine, 5-hydroxytryptamine and bradykinin at doses which were ineffective when injected alone. This synergistic effect was abolished by treatment with either meclizine, methysergide, Hoe 140 or des-Arg⁹-[Leu⁸]-bradykinin. Our findings show that local thermal hyperalgesia is a feature of allergen-evoked inflammation, and that a synergistic interaction among bradykinin, 5-hydroxytryptamine and histamine plays a critical role in this phenomenon.

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1. Introduction

Acute allergic reactions result from the immediate release of proinflammatory substances when an allergen interacts with immunoglobulin E (IgE) antibodies anchored on the surface of mast cells or basophils, leading to the cross-linking of IgE high-affinity receptors (FcεR1) (Busse and Lemanske, 2001; Kay, 2001). Therefore, in atopic patients, exposure of the skin, nose, airways or intestinal tract to allergen challenge causes inflammation within minutes, a phenomenon frequently associated with both tissue damage and nerve injury (Williams and Galli, 2000). Although common features of the inflammatory process in general include redness, swelling, and pain, there have been doubts concerning the putative implication of pain mechanisms in allergic conditions.

Allergic lesions including urticarial wheals and passive cutaneous anaphylaxis are marked by the production of itch instead of pain (Van Arman et al., 1968). Based on recent investigations of cutaneous nociception in atopic and non-atopic subjects, it has been proposed that itching could indeed be a distinct quality of nociception, also resulting from the stimulation of neuronal receptors in the superficial layers of the skin (Heyer and Hornstein, 1999). Recent studies have suggested that there is a down-regulation of C-fiber responsiveness to histamine, acetylcholine and vasoactive intestinal peptide in patients with urticaria or atopic eczema, giving support to the interpretation that the central nervous processing of itch is altered in these patients (Heyer et al., 1998; Rukwied and Heyer, 1999).

Sensitization of primary nociceptors often depends on the production and release of inflammatory mediators at the site of injury. The vasoactive amines, histamine and 5-hydroxytryptamine (5-HT), have classically been implicated in the processing of nociception in several

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inflammatory conditions (Besson, 1997). The involvement of these mediators in vascular changes, oedema formation and itching following allergen challenge is also well established (Kay, 2001). Tissue damage also leads to plasma leakage and subsequent activation of factors, which result in the release of kinins and other proinflammatory substances. Bradykinin has been critically implicated in the processing of nociceptive information and inflammation, its action being mediated by at least two major G-protein-coupled bradykinin receptors termed B₁ and B₂. The B₂ receptor is constitutively expressed on most cell types, whereas the B₁ receptor is not present in tissues under normal conditions but is often induced during inflammatory insults (Calixto et al., 2000).

Most of what is known about pain mechanisms is derived from experimental models of somatic nociception and inflammatory hyperalgesia. In general, inflammatory algometric tests are based on the analysis of the overt behavioral responses directly triggered by the inflammatory process, as in models of mouse abdominal constriction (Siegmund et al., 1957) and rat joint incapacitation (Tonussi and Ferreira, 1997). Inflammation-dependent overt behavioral responses can also be indirectly triggered by an exogenous secondary stimulus, as in the rat paw withdrawal test evoked by either pressure (Ferreira et al., 1978) or heating (Hargreaves et al., 1988). Since the mechanisms implicated in the processing of nociception vary and are certainly a reflection of the type of stimulus used, an evaluation of the putative proalgesic effects of the allergic inflammatory process could provide unique insights into the capability of the immune system to mediate events which can also result in nociception. Thus, in this study, we wanted to determine whether the immediate inflammatory response evoked by a plantar allergen challenge in rats could lead to a state of thermal hyperalgesia. The involvement of vasoactive amine and bradykinin was also investigated.

2. Materials and methods

2.1. Animals

All experiments were performed in male Wistar rats, weighing between 180 and 220 g, obtained from the breeding colony of the Oswaldo Cruz Foundation, Rio de Janeiro, Brazil. The animals were housed in groups of five and maintained on a 12-h light/dark cycle, with water and food ad libitum until use. The Ethics Committee for Care and Use of Laboratory Animals of The Oswaldo Cruz Foundation approved the experimental protocols employed in this study (License no. 0085-02).

2.2. IgE sensitization and hind paw challenges

In the group of IgE-sensitized rats, murine IgE anti-dinitrophenylated bovine serum albumin monoclonal anti-

body (0.5 µg/paw, 100 µl) and isotonic saline (100 µl) were injected intraplantarly into the left and right hind paws, respectively. In another group (sham-sensitized rats), 100 µl of isotonic saline was injected into both left and right hind paws. Sensitized and sham-sensitized left paws were then challenged with an intraplantar injection of allergen (dinitrophenylated bovine serum albumin, 0.1, 0.5 and 1 µg/paw, 100 µl) 24 h later, whereas the contralateral paws were injected with vehicle (isotonic saline, 100 µl). In another set of experiments, naive rats were injected intraplantarly with proinflammatory autacoids such as histamine (200–900 nmol/paw), 5-HT (40–140 nmol/paw) or bradykinin (5–50 nmol/paw) in a final volume of 100 µl. Co-stimulatory effects were evaluated by injecting subthreshold doses of these autacoids in different combinations, including histamine plus 5-HT, histamine plus bradykinin, bradykinin plus 5-HT and histamine plus 5-HT plus bradykinin. An equal volume of vehicle (isotonic saline, 100 µl) was injected into the contralateral paw for comparison.

2.3. Withdrawal responses to heat stimulus

This test was based on a previously reported method (Hargreaves et al., 1988) modified as described. Briefly, animals were individually placed on a hot plate (Eddy's Hot Plate) with the temperature adjusted to 51 °C, and the withdrawal response latency of each hind paw was determined at 15, 60, 180 and 360 min after immunological (allergen) or pharmacological (histamine, 5-HT and/or bradykinin) challenge. The heat source was maintained at constant intensity, which produced a stable withdrawal latency of approximately 8–10 s in vehicle-challenged paws. Hyperalgesia to heat was defined as a decrease in withdrawal latency and calculated as follows: $\Delta\text{paw withdrawal latency (s)} = \text{right paw withdrawal latency} - \text{left paw withdrawal latency}$.

2.4. Hind paw oedema measurement

The volumes of both hind paws of each rat were measured with a plethysmometer (Ugo Basile, Italy) at 15, 60, 180 and 360 min postchallenge. Hind paw swelling was expressed in microliters and calculated as follows: $\Delta\text{paw volume} = \text{left paw volume} - \text{right paw volume}$.

2.5. Treatments

Meclizine (30 mg/kg), methysergide (4 mg/kg) and cyproheptadine (4 mg/kg) were administered intraperitoneally 1 h before challenge. Hoe 140 (5 nmol/paw) and des-Arg⁹-[Leu⁸]-bradykinin (5 nmol/paw) were co-injected with the intraplantar stimuli. All drugs were dissolved in sterile saline, except meclizine that was dissolved in Tween 80 and further diluted with sterile isotonic saline. All solutions were prepared immediately before use.

2.6. Material

IgE anti-dinitrophenylated bovine serum albumin monoclonal antibody was kindly provided by Dr. Annie Provoust-Danon (Institut Pasteur-Paris, France) who, unfortunately, died in 1997. dinitrophenylated bovine serum albumin, methysergide, cyproheptadine, Hoe 140, des-Arg⁹-[Leu⁸]-bradykinin, histamine, 5-HT and bradykinin were purchased from Sigma (St. Louis, MO, USA). Meclizine was kindly provided by Pfizer (São Paulo, SP, Brazil).

2.7. Statistical analysis

All results are given as means \pm standard error of the mean and were analyzed statistically by one-way analysis of variance (ANOVA) followed by the Newman Keuls Student's *t*-test. *P* values of 0.05 or less were considered significant.

3. Results

3.1. IgE-mediated heat hyperalgesia and oedema

In adult Wistar rats, intraplantar injection of dinitrophenylated bovine serum albumin (0.1–1 $\mu\text{g/paw}$) into a hind paw previously sensitized with IgE anti-dinitrophenylated bovine serum albumin monoclonal antibody (0.5 $\mu\text{g/paw}$) led to a marked hyperalgesic response, as shown by the significant reduction in paw withdrawal latency on heat stimulation. The phenomenon set in very rapidly peaking from 15 to 60 min, diminishing thereafter to become unapparent 6 h postchallenge. For the lowest dose of dinitrophenylated bovine serum albumin (0.1 $\mu\text{g/paw}$), the thermal hyperalgesia was already unapparent within 60 min (Fig. 1A). In addition, IgE-mediated heat hyperalgesia occurred in parallel with a localized paw oedema response which also peaked within 15–60 min postchallenge, diminishing thereafter (Fig. 1B). Maximal hyperalgesic and oedematogenic responses were evoked by 0.5 $\mu\text{g/paw}$ dinitrophenylated bovine serum albumin which became the dose of choice for further experiments. It is noteworthy that intraplantar injection of dinitrophenylated bovine serum albumin (0.5 $\mu\text{g/paw}$) into sham-sensitized paws failed to modify the baseline levels of both paw withdrawal latency and volume (Fig. 1). Moreover, nothing happened to IgE-sensitized paws when isotonic saline replaced dinitrophenylated bovine serum albumin as challenge (data not shown).

3.2. Effect of histamine and 5-HT receptor antagonists

Intraperitoneal pretreatment of rats with the dual histamine and 5-HT receptor antagonist, cyproheptadine (4 mg/kg), 1 h before allergen challenge, clearly abolished both hyperalgesia and oedema responses evoked by dinitrophenylated bovine serum albumin (0.5 $\mu\text{g/paw}$), as shown in Fig. 2A and B, respectively. Thermal hyperalgesia was also entirely blocked by treatment with the selective histamine H₁ receptor antagonist, meclizine (30 mg/kg, i.p.) (Douglas, 1985), or methysergide (4 mg/kg, i.p.), which is recognized as a selective 5-HT₁, 5-HT₂ and 5-HT₇ receptor antagonist (Mylecharane, 1989) (Fig. 2A). The choice of doses was based on previous reports (Castro-Faria-Neto et al., 1992; Lima et al., 1996). In contrast, as shown in Fig. 2B, the allergen-evoked oedema response in animals pretreated with either meclizine or methysergide was not statistically different when compared with that of untreated animals, though with slightly lower values.

Intraplantar injection of histamine (200–900 nmol/paw) or 5-HT (40–140 nmol/paw) induced a dose-dependent thermal hyperalgesic response in the rat plantar test, as shown in Fig. 3. Table 1 shows that meclizine prevented the hyperalgesia caused by histamine (900 nmol/paw) without modifying the hyperalgesia caused by 5-HT (140 nmol/paw). In contrast, methysergide inhibited the effect of 5-HT but was ineffective against histamine, while cyproheptadine prevented the hyperalgesic effect of both agonists.

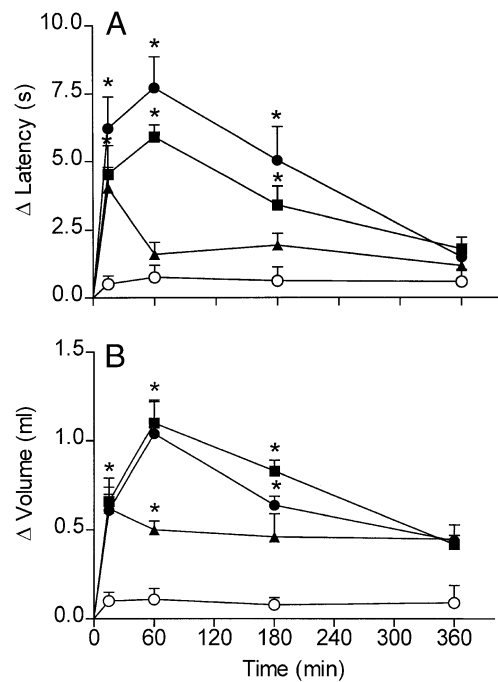


Fig. 1. Time course of thermal hyperalgesia (A) and oedema (B) caused by intraplantar challenge with increasing doses of dinitrophenylated bovine serum albumin (0.1–1 $\mu\text{g/paw}$) into sites passively sensitized with IgE anti-dinitrophenylated bovine serum albumin (0.5 $\mu\text{g/paw}$) 24 h before. In the negative control group, dinitrophenylated bovine serum albumin (0.5 $\mu\text{g/paw}$) was injected into paws pretreated with isotonic saline instead of IgE anti-dinitrophenylated bovine serum albumin. Closed and open symbols represent sensitized and sham-sensitized groups, respectively. Values are the means \pm S.E.M. for at least six animals. **P* \leq 0.05, significantly different from the negative control group.

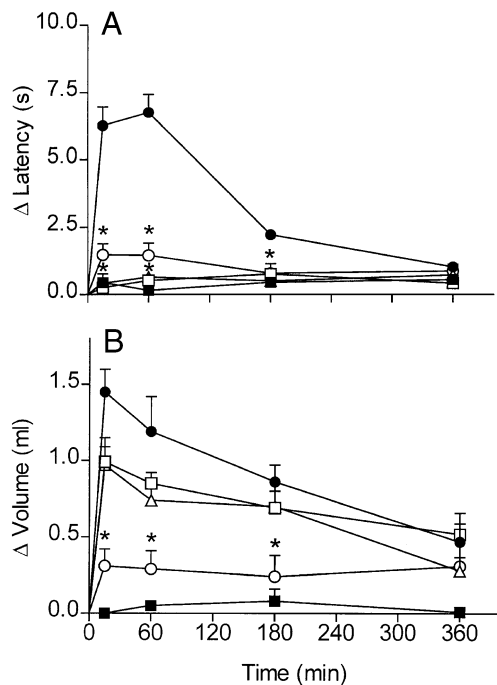


Fig. 2. Effect of meclizine (30 mg/kg, i.p.), methysergide (4 mg/kg, i.p.) and cyproheptadine (4 mg/kg, i.p.) on thermal hyperalgesia (A) and oedema (B) triggered by intraplantar challenge with dinitrophenylated bovine serum albumin (0.5 µg/paw) after IgE sensitization. Values are the means \pm S.E.M. for at least six animals. * $P \leq 0.05$, significantly different from the positive control group.

3.3. Effect of B_1 and B_2 bradykinin receptor antagonists

Allergen-evoked hyperalgesia was also clearly sensitive to treatment with bradykinin receptor antagonists. Local treatment with either the B_2 antagonist, Hoe 140 (Hock

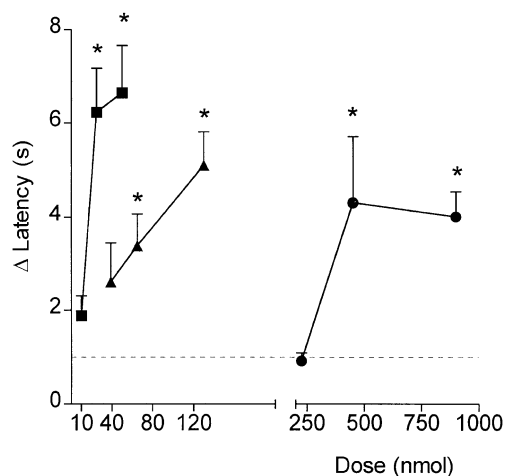


Fig. 3. Hyperalgesic effect of intraplantar injections of histamine (200–900 nmol/paw), 5-HT (40–140 nmol/paw) and bradykinin (10–50 nmol/paw). Values are the means \pm S.E.M. for at least six animals. * $P \leq 0.05$, significantly different from the control group, which received an intraplantar injection of isotonic saline instead of histamine, 5-HT or bradykinin (dashed line).

Table 1

Effect of meclizine, methysergide and cyproheptadine on thermal hyperalgesia evoked by either histamine or 5-HT intraplantar challenge

Agonist	Treatment	Time postchallenge		
		15 min	1 h	3 h
Histamine	Vehicle	4.36 \pm 0.95	3.04 \pm 0.37	1.54 \pm 0.56
	Cyproheptadine	0.60 \pm 0.37 ^a	0.80 \pm 0.19 ^a	0.74 \pm 0.21
	Meclizine	0.83 \pm 0.26 ^a	0.90 \pm 0.49 ^a	0.77 \pm 0.60
5-HT	Methysergide	4.38 \pm 0.59	2.74 \pm 0.12	2.08 \pm 0.60
	Vehicle	4.20 \pm 0.97	7.00 \pm 0.84	3.24 \pm 0.77
	Cyproheptadine	0.70 \pm 0.36 ^a	0.45 \pm 0.52 ^a	0.64 \pm 0.54
	Meclizine	6.25 \pm 1.03	6.95 \pm 0.50	2.70 \pm 0.62
	Methysergide	0.94 \pm 0.54 ^a	1.00 \pm 0.57 ^a	0.86 \pm 0.25

Meclizine (30 mg/kg), methysergide (4 mg/kg) and cyproheptadine (4 mg/kg) were administered intraperitoneally 1 h before histamine (900 nmol/paw) or 5-HT (140 nmol/paw) intraplantar challenge. Values are the means \pm S.E.M. for at least six animals.

^a $P < 0.05$ as compared to the respective untreated control group.

et al., 1991; Wirth et al., 1991; Feletou et al., 1994; Stewart et al., 1999), or B_1 antagonist, des-Arg⁹-[Leu⁸]-bradykinin (Regoli and Barabe, 1980) (5 nmol/paw), prevented the reduction in paw withdrawal latency to heat stimulation when administered in combination with dinitrophenylated bovine serum albumin (Fig. 4A). The choice of dose and route of administration were based on previous reports (Coelho et al., 1997; Bandeira-Melo et al., 1999).

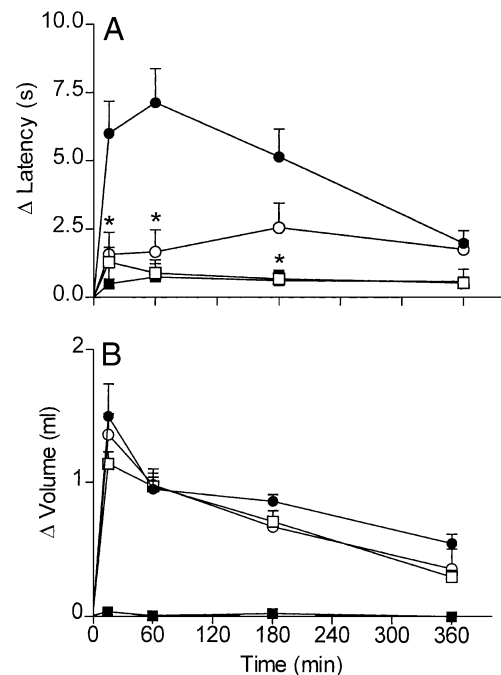


Fig. 4. Effect of intraplantar co-administration of the B_2 receptor antagonist, Hoe 140 (5 nmol/paw) or B_1 receptor antagonist, des-Arg⁹-[Leu⁸]-bradykinin (5 nmol/paw) on thermal hyperalgesia (A) and oedema (B) triggered by intraplantar challenge with dinitrophenylated bovine serum albumin (0.5 µg/paw) in sensitized animals. In the positive control group (closed symbol), bradykinin receptor antagonists were omitted. Values are the means \pm S.E.M. for at least six animals. * $P \leq 0.05$, significantly different from the positive control group.

Table 2
Effect of treatment with either Hoe 140 or des-Arg⁹-[Leu⁸]-bradykinin on bradykinin-induced thermal hyperalgesia

Agonist	Treatment	Time postchallenge		
		15 min	1 h	3 h
Bradykinin	None	4.88 ± 1.03	6.18 ± 1.75	3.87 ± 0.59
	Hoe 140	1.62 ± 0.87 ^a	0.83 ± 0.38 ^a	0.68 ± 0.29 ^a
	des-Arg ⁹ -[Leu ⁸]-bradykinin	3.33 ± 0.51	1.67 ± 0.70 ^a	0.70 ± 0.34 ^a

Hoe 140 and des-Arg⁹-[Leu⁸]-bradykinin (5 nmol/paw), bradykinin B₂ and bradykinin B₁ receptor antagonist were co-injected with bradykinin (50 nmol/paw) in a final volume of 100 µl. Values are the means ± S.E.M for at least six animals.

^a $P < 0.05$ as compared to the untreated control group.

Stimulation with increasing doses of bradykinin (10–50 nmol/paw) also led to a state of dose-dependent hyperalgesia (Fig. 3), a phenomenon clearly sensitive to treatment with either B₁ or B₂ receptor antagonists (Table 2). On the other hand, like meclizine and methysergide, both Hoe 140 and des-Arg⁹-[Leu⁸]-bradykinin failed to modify the paw

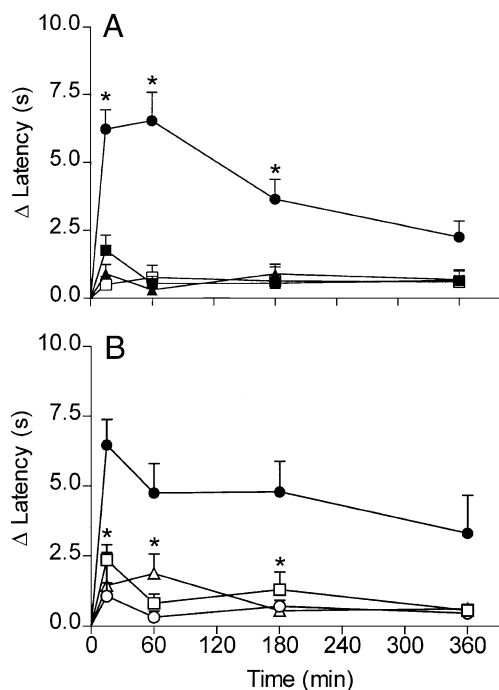


Fig. 5. (A) Analysis of thermal hyperalgesia caused by intraplantar injection of a mixture of subthreshold doses of histamine (45 nmol/paw) and 5-HT (2.6 nmol/paw) (closed circle). Effect of histamine (45 nmol/paw) or 5-HT (2.6 nmol/paw) injected alone is indicated by open symbols. Values are the means ± S.E.M. for at least six animals. * $P \leq 0.05$, significantly different from the group stimulated with histamine alone or 5-HT alone. (B) Effect of meclizine, methysergide and Hoe 140 on thermal hyperalgesia evoked by intraplantar histamine and 5-HT dual challenge (45 and 2.6 nmol/paw, respectively). In the positive control group (closed circle), isotonic saline replaced receptor antagonists as treatment. Values are the means ± S.E.M. for at least six animals. * $P \leq 0.05$, significantly different from the positive control group.

oedema triggered by allergen challenge (Fig. 4B), adding support to the interpretation that oedema and hyperalgesia are regulated differently in this model.

3.4. Synergism between histamine, 5-HT and bradykinin in causing thermal hyperalgesia

While trying to clarify why histamine, 5-HT and bradykinin receptor antagonists were equally able to prevent allergen-evoked hyperalgesia, we investigated the possibility that histamine, 5-HT and bradykinin act synergistically in order to sensitize the nociceptor. As shown in Fig. 5A, the combination of histamine (45 nmol/paw) and 5-HT (2.6 nmol/paw) induced a marked reduction in the paw withdrawal latency under conditions where they failed to evoke any effect when injected alone. As shown in Fig. 5B, the hyperalgesia triggered by the combination of histamine and 5-HT was clearly abolished by treatment with meclizine, methysergide or Hoe 140.

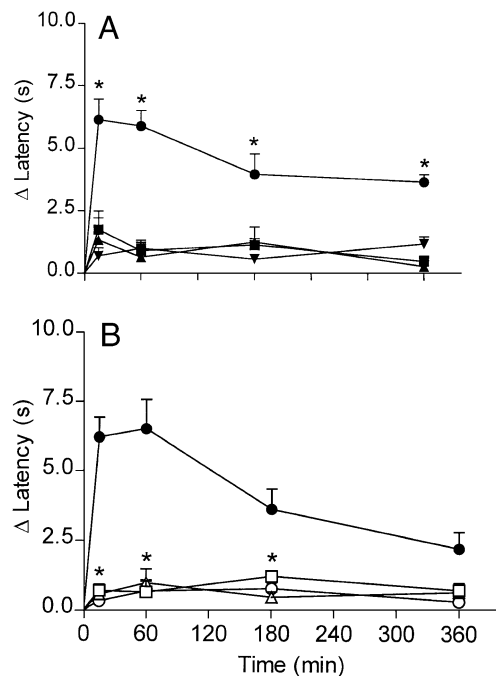


Fig. 6. Triple synergism between histamine, 5-HT and bradykinin reduces even more the doses required to cause hyperalgesia. (A) Time course of thermal hyperalgesia caused by intraplantar triple co-injection of histamine, 5-HT and bradykinin (22.5, 1.3 and 0.5 nmol/paw, respectively). Effect of co-injections of histamine and 5-HT (22.5 and 1.3 nmol/paw, respectively), histamine and bradykinin (22.5 and 0.5 nmol/paw, respectively) and bradykinin and 5-HT (0.5 and 1.3 nmol/paw, respectively) is indicated by open symbols. Values are the means ± S.E.M. for at least six animals. * $P \leq 0.05$, significantly different from the group stimulated with histamine–5-HT, histamine–bradykinin and bradykinin–5-HT dual challenges. (B) Effect of meclizine, methysergide and Hoe 140 on thermal hyperalgesia evoked by intraplantar histamine, 5-HT and bradykinin triple challenge (22.5, 1.3 and 0.5 nmol/paw, respectively). In the positive control group (closed circle), isotonic saline replaced receptor antagonists as treatment. Values are the means ± S.E.M. for at least six animals. * $P \leq 0.05$, significantly different from the positive control group.

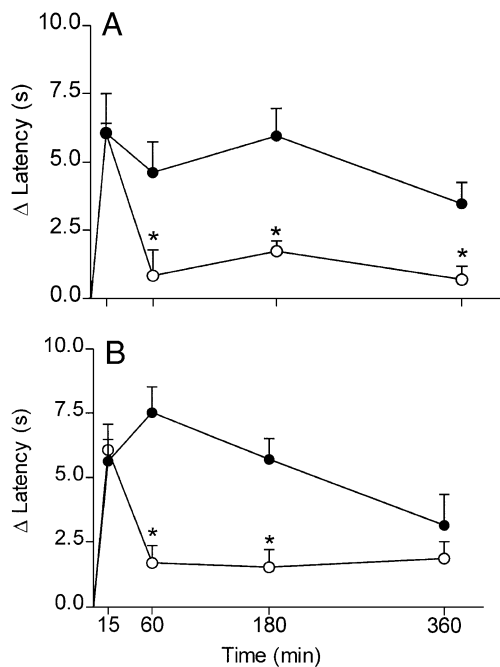


Fig. 7. Kinetics of the protective effect of B_1 receptor antagonist des-Arg⁹-[Leu⁸]-bradykinin (5 nmol/paw) on thermal hyperalgesia caused by either histamine and 5-HT dual challenge (A) or histamine, 5-HT and bradykinin triple challenge (B). Open and closed symbols represent treated and untreated groups, respectively. Values are the means \pm S.E.M. for at least six animals. * $P \leq 0.05$, significantly different from the untreated control group.

We also found that the triple challenge with histamine, 5-HT and bradykinin, at doses of 22.5, 1.3 and 0.5 nmol/paw, respectively, led to an intense hyperalgesic response under conditions where dual challenge with histamine plus 5-HT (22.5 and 1.3 nmol/paw, respectively), histamine plus bradykinin (22.5 and 0.5 nmol/paw, respectively) and bradykinin plus 5-HT (0.5 and 1.3 nmol/paw, respectively) was ineffective (Fig. 6A). As shown in Fig. 6B, the reduction in paw withdrawal latency triggered by the triple challenge was clearly prevented by treatment with meclizine, methysergide or Hoe 140, mimicking the situation observed after allergen challenge. Furthermore, both double and triple synergism, involving histamine and 5-HT, and histamine, 5-HT and bradykinin, respectively, were also clearly sensitive to the B_1 -receptor antagonist, des-Arg⁹-[Leu⁸]-bradykinin (Fig. 7). Similar to what was observed in case of bradykinin-evoked hyperalgesia, the blockade was seen from 60 to 180 min but not at 15 min postchallenge.

4. Discussion

The major findings of the present study are: (i) IgE-mediated acute inflammation in the rat plantar test is marked by parallel development of paw oedema and thermal hyperalgesia; (ii) allergen-evoked hyperalgesia is clearly sensitive

to blockade by receptor antagonists of histamine, 5-HT and bradykinin; (iii) the combination of histamine, 5-HT and bradykinin synergistically stimulated thermal hyperalgesia, and the selective blockade of one of the agonists of the triple synergism is enough to abolish the entire response. These findings support the interpretation that hyperalgesia is indeed a feature of allergic inflammation, and that the phenomenon is strongly dependent on the synergism between vasoactive amines and bradykinin.

Although little information is available on allergen-evoked nociception, patients with allergies are characterized by a higher psychoemotional response to pain stimuli than patients without the accompanying pathology (Stosh et al., 1991). Moreover, inflammatory mediators supposed to play a key role in the development of allergic inflammation, including histamine, 5-HT, bradykinin, leukotriene, platelet activating factor and interleukin-4 (Kay, 2001), are also well established hyperalgesic agents (Besson, 1997). These observations suggest that hyperalgesia and nociceptive pain should be considered in the context of allergic inflammation, possibly being implicated in the response to allergen in several allergic dysfunctions including rhinitis and food allergy. In this study, the paw withdrawal reflex threshold following plantar thermal stimulus was used as a measure of nociceptive response in rats passively sensitized with IgE anti-DNP mAb. The threshold in the area of local tissue damage created by the dinitrophenylated bovine serum albumin challenge was about 5–7 s lower than the threshold of the sham-sensitized contralateral paw, also injected with dinitrophenylated bovine serum albumin. This indicated that allergen challenge is capable of triggering a hyperalgesic response, which was very rapid in onset and occurred in parallel with oedema, both peaking within 15–60 min postchallenge, diminishing thereafter. Moreover, our data showed that the two phenomena were regulated separately since the allergen-evoked hyperalgesia but not the oedema was abolished by individual treatment with either histamine or 5-HT receptor blockade, whereas dual blockade with cyproheptadine was required for the inhibition of oedema. These findings are consistent with the interpretation that oedema by itself does not necessarily cause pain, and indicate that histamine and 5-HT are implicated in both oedema and hyperalgesia associated with allergic inflammation.

Sensitization of the pain receptors by histamine and 5-HT had been described in early studies (Sicuteri et al., 1965). Here, we confirmed that the doses of histamine and 5-HT required to induce rat paw hyperalgesia were relatively high but could be markedly reduced when the agonists were injected in combination, giving support to a recurrent suggestion in the literature that the synergism between histamine and 5-HT (Gilfoil and Klavins, 1965; Parada et al., 2001) indeed plays a critical role in several inflammatory conditions. Similarly to what was observed following allergen challenge, it was possible to prevent hyperalgesia caused by the vasoactive amine dual challenge using either

specific histamine or 5-HT receptor blockade. The data suggest that these agonists act in parallel to produce hyperalgesia, both being equally required in this case. It is noteworthy that under our current conditions, paw hyperalgesia caused by the dual histamine and 5-HT challenge was also sensitive to the bradykinin B₂ receptor antagonist, Hoe 140, suggesting that during inflammation triggered by the combination of histamine and 5-HT there is also the pivotal contribution of bradykinin, whose generation, in that case, might result from the activation of plasma precursors (kininogens) following plasma leakage evoked by the vasoactive amines.

Bradykinin is a potent mediator of inflammation and may play a greater role in mediating the early phase of the antigen-induced allergic response (Hogan et al., 1997). The pathophysiological role of this nonapeptide in experimental allergic conditions is strongly supported by the finding of increased amounts of bradykinin in the nasal cavity of atopic patients (Proud et al., 1983) and ovalbumin-sensitized guinea pigs (Shirasaki et al., 1989), observed immediately after nasal allergic challenge. According to results of these studies, the presence of kinin was highly correlated with that of histamine and with clinical symptoms, including local vascular permeability increase. A recent report has demonstrated that Hoe 140, a bradykinin B₂ receptor antagonist, abolishes the hyperresponsiveness and nasal eosinophilia induced by antigen (Turner et al., 2001). The protective effect of B₂ receptor antagonists is also evidenced in other experimental models of allergic inflammation including allergen-mediated rat pleurisy (Bandeira-Melo et al., 1999) and late response in the allergic sheep model (Abraham et al., 1991). The relative involvement of the two bradykinin receptors (B₁ and B₂) concerning inflammatory hyperalgesia is still a matter of active debate in the literature. An early study (Davis and Perkins, 1994a,b) demonstrated that following intraarticular injection of Freund's complete adjuvant both bradykinin B₁ and B₂ receptor mechanisms are involved in the accompanying hyperalgesia. These data are in line with those reported by (Petersen et al., 1998) showing that the expression of bradykinin B₁ receptors is induced and the expression of bradykinin B₂ receptors is enhanced in sensory neurons after mechanical nerve injury. Yet, according to two reports (Poole et al., 1999; Banik et al., 2001), there is a predominant role for bradykinin B₂ receptors in the mediation of hyperalgesic responses, whereas evidence for the participation of bradykinin B₁ rather than bradykinin B₂ receptor in Freund's adjuvant-induced nociceptive responses has also been provided (Ferreira et al., 2001). In order to assess the effect of selective blocking of the bradykinin B₁ or B₂ receptor on allergen-evoked hyperalgesia, des-Arg⁹-[Leu⁸]-bradykinin (bradykinin B₁ receptor antagonist) and Hoe 140 were administered to sensitized rat paws in association with the allergen challenge. Our data showed that Hoe 140 as well as des-Arg⁹-[Leu⁸]-bradykinin prevented the reduction in paw withdrawal latency caused by either bradykinin or

allergen challenge. These findings strongly suggest that the kinin system is indeed implicated in the process of anaphylactic hyperalgesia, probably in a mechanism driven by both B₁ and B₂ bradykinin receptors.

The above considerations made it reasonable to assume that during allergic inflammation, there is a sensitization of pain receptors that ensues after mast cell degranulation and plasma leakage, and is accounted for by the synergistic interaction of a set of inflammatory mediators including histamine, 5-HT and bradykinin. Consistent with the idea of multiple and synergistic mediation in the processing of nociception, we hypothesized that the selective blockade of one of the mediators involved in the synergism would be enough to stop the hyperalgesic process, as predicted by the pharmacological modulation of allergen-evoked hyperalgesia. Our data indicated that a mixture of histamine, 5-HT and bradykinin at very low concentrations (40-, 100- and 100-folds lower than the respective dosages required for an equivalent response when injected alone) led to a hyperalgesic response comparable to that noted in allergen-challenged paws. Under such conditions, the dual challenges with histamine plus 5-HT, histamine plus bradykinin and bradykinin plus 5-HT were ineffective. Indeed, the response evoked by the triple challenge was clearly prevented by either histamine, 5-HT or bradykinin receptor blockade, including B₁ and B₂ receptor antagonists, mimicking the situation observed following allergen challenge. It is noteworthy that in the case of double and triple synergism, as was observed in the case of hyperalgesia evoked by bradykinin alone, the protective effect of the bradykinin B₁ receptor antagonist does not appear until 60 min postchallenge, suggesting that bradykinin B₁ receptors are being up-regulated under such conditions. Further experiments are now underway in order to clarify this possibility.

In conclusion, a state of thermal hyperalgesia is present in the area of tissue damage created by allergen challenge. The phenomenon is shown to be independent of the concomitant oedematogenic response and is critically mediated by the synergistic interaction of at least three autacoids—histamine, 5-HT and bradykinin. Thus, it is likely that the IgE-mediated mechanisms can also result in nociceptive events under various pathophysiological conditions.

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